ON THE CONFORMATION OF THE ACETYLCHOLINE RECEPTOR PROTEIN FROM *TORPEDO NOBILIANA*

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1. Introduction

It is well established that acetylcholine acts as a regulatory ligand controlling selective increase in membrane permeability to cations [1]. This permeability change is thought to be mediated by a minimum of two distinct structural elements [2]: a 'receptor' protein which recognizes cholinergic agonists and an 'ionophore' that accounts for the selective transition of ions. The latter could be a region of the receptor protein or may constitute a separate but tightly coupled entity [2]. AChR‡ from several species has been extensively purified by several laboratories [3–9] and its molecular properties are currently under investigation. However, no information is available on the receptor conformation.

This report describes for the first time the far ultraviolet CD spectrum of AChR. A detailed analysis of the resolved spectrum indicates that the *Torpedo nobiliana* AChR contains a particularly high content of ordered secondary structure, e.g., about 34% α-helix and 29% β-structure. The amino acid composition of the receptor is also reported and the overall polarity and hydrophobicity is typical of soluble proteins.

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‡ *Abbreviations:* AChR, acetylcholine receptor protein; CD, circular dichroic.

2. Materials and methods

The *T. nobiliana* AChR was purified by affinity chromatography [3], concentrated using a DEAE-Sephadex column equilibrated with 50 mM K^+-phosphate buffer, pH 7.0, 0.1% Emulphogene BC-720, and eluted with the same buffer containing 0.5 M NaCl. The first ml of effluent was diluted approximately two-fold with the eluting buffer to give a 114 μg protein/ml solution which was used for all studies. The assay for AChR activity and the preparation of neurotoxins were as previously reported [3]. The specific activity of the purified AChR was 80 000 daltons protein bound per cobratoxin molecule [3]. Amino acid analysis [10] was carried out on a Beckman Model 121 amino acid analyzer with a norleucine internal standard. Protein was determined by the Lowry method [11] with a bovine serum albumin standard. Absorption and CD spectra were obtained with an Hitachi–Perkin–Elmer EPS-3T spectrophotometer and a Cary 60 spectropolarimeter (with a CD attachment), respectively.

3. Results

3.1. *Amino acid composition*

The preliminary amino acid compositions of AChR of *T. nobiliana, E. electricus* [5,8] and *T. marmorata* [4,9] are given in table 1. Each has a basic and acidic amino acid content of 11–12% and 21–23%, respectively, the latter of which does not include the amidated forms. Our results show the presence of tryptophan which was also found in the receptor from *T. marmorata* [4] and *E. electricus* [8]. Noteworthy is the consistency and close agreement

Table 1
Amino acid composition* of the acetylcholine receptor protein from *Torpedo nobiliana* and other species

	T. nobiliana	*T. marmorata* [4]	*T. marmorata* [9]	*E. electricus* [5]	*E. electricus* [8]
Lysine	4.5	6.1	5.0	4.6	6.3
Histidine	2.5	2.1	2.3	2.2	2.5
Arginine	3.7	3.5	3.7	4.2	4.2
Tryptophan	1.5	2.1	–†	0	2.4
Aspartic acid	12.2	11.8	12.5	11.4	9.8
Threonine	6.8	6.3	6.0	5.6	6.0
Serine	6.4	7.1	8.2	6.2	8.2
Glutamic acid	9.7	10.7	8.8	10.2	9.0
Proline	7.1	6.2	5.6	5.7	6.7
Glycine	5.0	6.4	5.0	5.9	4.8
Alanine	4.5	6.0	5.0	5.8	5.4
Half-cystine	2.8**	2.0	–‡	2.0	1.7
Valine	6.2	5.5	7.5	8.6	6.9
Methionine	1.6	1.7	2.6	2.0	3.4
Isoleucine	6.2	5.2	7.5	6.4	8.1
Leucine	10.2	9.3	10.2	10.5	10.7
Tyrosine	4.2	3.6	3.7	4.0	3.8
Phenylalanine	4.2	4.4	4.6	5.7	5.1
Glucosamine	2.0	–	–	–	–

* The data are reported as mole %. It is a pleasure to thank Lilah Clack for the analysis of *T. nobiliana.*
** Represents the sum of half-cystine and cysteic acid.
† Not analyzed.
‡ Present but not quantitated.

of the amino acid compositions of AChR from various species. At least over this limited phylogenetic spectrum a common structure appears to have been preserved.

Due to the membranous environment of AChR in vivo, it is of interest to determine its polarity and hydrophobic nature relative to other proteins. Protein polarity has been estimated by the per cent of the polar residues Asp, Asn, Glu, Gln, Lys, Ser, Arg, Thr, and His [12]. By this criterion the *T. nobiliana* AChR is 46% polar which is very similar to the value of 47% reported for the *E. electricus* AChR [13]. Analysis of a large number of proteins showed that most of the soluble proteins had polarities of 47 ± 6%, whereas many of the membrane proteins had polarities less than 40% [12]. Using a somewhat more quantitative approach involving transfer free energies (e.g., from water to organic solvent) of the various amino acid side chains [14,15], we find a hydrophobicity of 1236 cal for the *T nobiliana* AChR. This is only slightly greater than the average value reported for several soluble proteins. For example, hemoglobin, cytochrome c, insulin, and lactate dehydrogenase have respective hydrophobicities of 1158, 1103, 1157 and 1127 ± 10% cal [14].

Glucosamine is present in the *T. nobiliana* AChR (about 3.8% w/w protein), however, the data are too tentative to conclude that AChR is a glycoprotein. Carbohydrate has also been reported in AChR of *E. electricus* [13] and hexosamine has been observed in *T. marmorata* [4], but its presence was attributed to an artifact arising from the affinity absorption procedure.

3.2. *Absorption spectroscopy*

The ultraviolet absorption spectrum of AChR is shown in fig. 1 and is characterized by an absorption maximum at 281 nm. The presence of tryptophan is clearly indicated from the spectrum by the shoulder at 290 nm and the shoulder at 260 nm is characteristic of phenylalanine.

3.3. *CD spectroscopy*

The resolved [16] far ultraviolet CD spectrum of

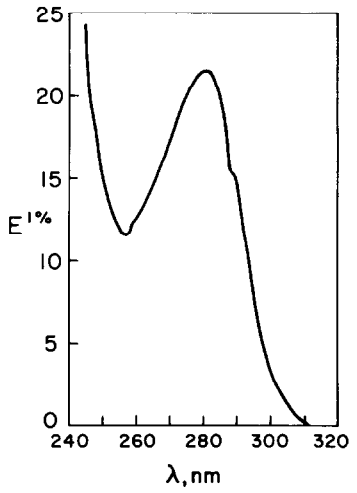

Fig. 1. The absorption spectrum of AChR in 0.1% Emulphogene BC-720 and the buffer described in the text. Light scattering corrections (e.g., $A_{360}/A_{281} = 0.11$) were made by extrapolating the apparent absorbance (log A vs log λ) between 320–360 nm to the region 240–320 nm. The protein concentration was taken as the average from amino acid analysis and the Lowry method; agreement was within 1%. The absorbance at the 281 nm maximum for a 1% solution (1 cm) is 21.5.

AChR is shown in fig. 2. The spectrum between 205–245 nm is characterized by two negative extrema at 207.5 and 215 nm with mean residue ellipticities of −13 300 and −14 670 deg · cm^2/dmole, respectively. The spectrum can be fit to within ± 3% at all

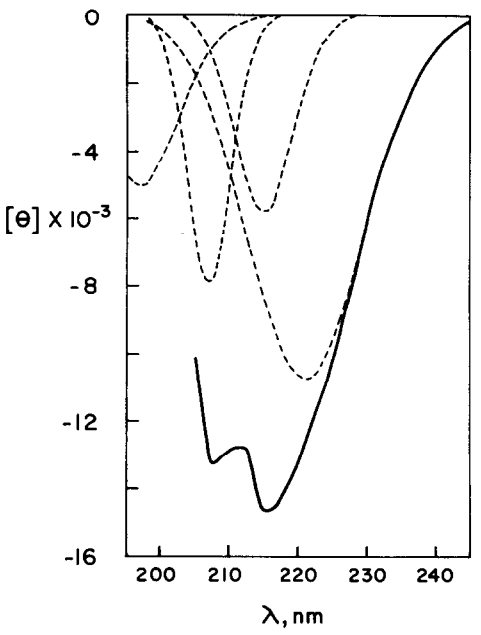

Fig. 2. The far ultraviolet CD spectrum of AChR (——) at ambient temperature and the resolved gaussian bands (– – –). AChR was in the buffer containing 0.1% Emulphogene BC-720. The experimental spectrum shown represents the average of several sample and baseline scans. Reproducibility was good and under the experimental conditions used (1.0 mm pathlength-fused silica cell, 3 sec time constant, and scale expansions of 2 millidegrees/inch and 7.5 nm/inch) the signal to noise ratio was quite high, e.g., at 215 nm the baseline noise was ± 0.8 millidegrees and the signal was 14 ± 1 millidegrees. The mean residue ellipticity in units of deg·cm^2/dmole was calculated with a mean residue mol. wt. of 113.8 which was determined from the data in table 1.

Table II

Mean residue rotational strengths of the far ultraviolet circular dichroic bands of the *T. nobiliana* AChR and major protein conformers

AChR		Proteins[a]		Transition	Conformer
(λ_0, nm)	(R)[b]	(λ_0, nm)	(R)[b]		
221	−7.17	222	−21.0	n–π*	α-Helix
215	−1.99	215	− 7.77	n–π*	β-Structure
207	−2.11	208	− 6.26	π–π*	α-Helix
197	−2.47	197	− 7.27	π–π*	Aperiodic

[a] These data are from [19] and were resolved from the average CD spectra for the α-helical, β-structure, and aperiodic conformations as determined by Chen et al. [18].

[b] The rotational strength (R, in 10^{-42} cgs units) of each resolved gaussian band was obtained using the following relationship: $R \simeq (1.234 \times 10^{-42})$. $[\theta^\circ] \cdot \Delta/\lambda_0$, where $[\theta^\circ]$ and λ_0 denote the magnitude and wavelength of the extremum, and Δ is the bandwidth.

wavelengths by 4 resolved gaussian bands which are described in table 2. The 221 nm and 215 nm bands are assigned to the $n-\pi^*$ transition of the peptide chromophore in α-helical regions and β-structure, respectively. The lower wavelength bands probably arise from the $\pi-\pi^*$ transition of the peptide chromophore; however, the overlap of several large positive and negative bands in this region of the spectrum prohibits definitive resolution unless the experimental spectrum extends to about 185 nm.

We have used two methods [17] to estimate the type and amount of ordered secondary structure. One method involves a least-squares fit of AChR ellipticity from 207 to 243 nm to a linear combination of α-helical, β-structure, and aperiodic contributions [18]. The second method is based on a comparison of the rotational strengths of the 221 and 215 nm bands with the corresponding values [19] for the pure conformers [18].

These two methods gave the following values: 34% α-helix, 32% β-structure, and 34% aperiodic conformation by the least squares analysis, and 34% α-helix, 26% β-structure and thus 40% aperiodic conformation by the ratios of rotational strengths of the 221 and 215 nm bands. These results suggest that AChR contains a particularly high content of ordered secondary structure, e.g., 34% α-helix and 29 ± 3% β-structure.

No attempt has been made to correct these estimates of secondary structure for the contributions of aromatic residues to the far ultraviolet CD spectrum and for the effects of light scattering. Generally, light scattering tends to reduce the magnitude of the CD extrema [16] and aromatic residues in model compounds exhibit positive ellipticity above about 215 nm (L. A. Holladay and D. Puett, unpublished). These considerations lead to the conclusion that our estimates on the amount of α-helix and β-structure probably represent minimal values.

The effects of various cholinergic analogs on the CD spectrum of AChR are currently under investigation. Tentative data with carbamylcholine indicate that even low concentrations (e.g., 1–4 μM which corresponds to low binding saturation) alter both the magnitude of the apparent light scattering at 360 nm and the CD spectrum in the vicinity of the 207.5 nm extremum. These spectral changes noted at low degrees of saturation give impetus to extending this work to saturating levels of the cholinergic ligand.

4. Discussion

The *T. nobiliana* AChR has gross polarity and hydrophobicity characteristics like those of many soluble proteins. Unfortunately, these considerations provide no information on the distribution of hydrophobic residues. The portion of AChR containing the cholinergic binding site is most certainly located on the membrane surface and exposed to the synaptic cleft. However, the solubility properties of AChR indicate extensive hydrophobic regions and, thus, part of AChR may penetrate the membrane. It is of immense interest to determine if the hydrophilic and hydrophobic residues are randomly distributed along the polypeptide backbone or are clustered in distinct regions. The answer to this important issue must await additional chemical studies.

Hucho and Changeux [20] have suggested that *E. electricus* AChR denotes a complex of approximate mol. wt. 230 000 consisting of multiple subunits of two distinct molecular weight classes. They have developed a model with three (probably identical) subunits which bind the cholinergic ligand, i.e., the receptors, and which are in intimate contact with different subunits involved in ion transport, i.e., the ionophores. This model is characterized by a channel, formed by the arrangement of the subunits, through which ions are transported [21]. Thus, within the context of this model, the AChR subunits may exhibit hydrophilic and hydrophobic domains or inverted conformations [13,21].

Our finding of a high content of ordered secondary structure in AChR is of interest both in assessing the conformation of AChR and in comparative conformational studies. For example, other studies have shown that the average α-helicity of mitochondrial membrane proteins is about 27% and there is not a large amount of β-structure [16]. Also, we found that the intrinsic mitochondrial membrane proteins are more helical than the extrinsic proteins [16].

Frequently, β-structure in proteins is associated with a relatively compact structural core and, thus, much of AChR may exist in an extensively folded conformation, i.e., globular-like, reminiscent of soluble proteins. Indeed, the electron micrographs of AChR tend to support this model [13,22].

Many speculations can be made regarding the role and locations of the α-helical and β-structure regions.

For example, the α-helical regions may exhibit conformational amphipathic properties [23,24] in which hydrophilic side-chains are located mainly on one surface area of an α-helix while the other is comprised primarily of hydrophobic side-chains. An array of such structures within the ionophore would constitute an excellent ion channel from a thermodynamic viewpoint since the hydrophilic groups would be in contact with an aqueous phase and the hydrophobic groups would be located within an apolar environment arising either from the protein or the lipid moiety. Regions of β-structure may, of course, be involved in forming the ion channel, but it would seem that their predominant location would be within the globular portions of the protein.

Current studies are in progress to determine if the conformation of AChR is altered during interaction with cholinergic ligands.

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